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To determine whether single presentation of light or physical activity can phase shift the human circadian clock, 8 young male subjects were subjected to the following experimental protocol. Following entrainment to a fixed sleep-wake and light-dark cycle for one week, each subject underwent 3 separate studies: one baseline study in which measurements of circadian phase positions were performed under "constant routine" conditions (i.e. constant wakefulness in recumbent position under constant dim light with constant caloric intake for 42 hrs), and two studies in which each subject was exposed to a 3-h session of either bright light (5000 Lux) or physical activity (exercise on a stationary arm-and-leg exerciser) during the "constant routine" regimen. In order to estimate accurately circadian phase positions, 8 overt rhythms were monitored in each subject: plasma cortisol, plasma TSH, plasma melatonin, plasma glucose, plasma C-peptide, core temperature, total activity and mental performance. The immediate phase shifting effects of bright light or exercise were measured on the monitored rhythms on the first day following stimulus presentation. Preliminary analysis of currently available data indicate that both light and exercise resulted in a phase advance of approximately one hour.

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## STATEMENT OF WORK

Major advances in our understanding of the mammalian circadian clock system have recently emerged from animal studies. Indeed, a series of agents (e.g. benzodiazepines, protein synthesis inhibitors) or stimuli (e.g. increased locomotor activity, social stimuli) capable of affecting the phase and/or the period of the circadian pacemaker through pathways independent of light have been identified. Although diverse in nature, these newly recognized zeitgebers exhibit similar phase-response curves and therefore may exert their effects through a common input pathway into the clock. Despite the enormous implications of circadian rhythms for human health and disease, basic research on the control of circadian rhythmicity in man is lagging considerably behind studies in animals. Indeed, the importance of bright light as a zeitgeber in the human has only recently been recognized, and phase-shifting effects of single presentations of non-photic stimuli remain to be demonstrated. Methodological difficulties have hampered progress in the field. The major approach used by biologists to probe the circadian system, i.e. the measurement of phase-response curves in free-running animals maintained in constant conditions, is extremely cumbersome, costly and time-consuming to implement for human subjects. Furthermore, it remains to be determined if results obtained in humans maintained in temporal isolation for extended periods of time can be readily translated to the more natural condition where zeitgebers are always present in the environment. Finally, because overt rhythms which can be used as circadian markers are more prone to masking by behavioral inputs in humans than in laboratory animals, it has been difficult to determine with accuracy the status of the clock at any particular time.

Under the auspices of the present grant, we are using two novel procedures to determine in normal human subjects the phase-shifting effects of potential zeitgebers presented on the first day following entrainment. Our specific aims are:

1. to determine the magnitude and direction of immediate phase-shifts associated with a single exposure to a 3-hour pulse of either bright light or physical activity. Following a period of rigorous entrainment to a fixed sleep-wake and light-dark cycle, the pulse is presented at three different circadian times during a 24-hour cycle of "constant routine" (i.e. a regimen of constant wakefulness in recumbent position, constant dim light and constant caloric intake). Measurement of the resulting phase-shifts are performed under "constant routine" conditions on the first day following pulse presentation.

2. to estimate the magnitude and direction of stable phase-shifts associated with a single exposure to a 3-hour pulse of bright light or physical activity. The pulse is presented at three different circadian times on the first day following rigorous entrainment to a fixed light-dark and sleep-wake cycle. During the day of presentation and during the two following days, the subjects are maintained under an ultradian sleep-wake and light-dark schedule with a 4.5-hour period, i.e. well outside the range of

entrainment of circadian rhythmicity. Measurement of the phase-shifts are performed under "constant routine" conditions on the third day following pulse presentation. This 3-day protocol was designed as a compromise permitting the observation of the major part of the total shift while limiting the duration of the experiment.

To improve the reliability and robustness of our estimations of circadian phase positions and to increase our ability to discriminate peripheral effects on overt rhythms from central effects on the circadian clock, we monitor simultaneously five overt rhythms which are strongly dependent on circadian timing, i.e. the rhythms of plasma cortisol levels, plasma melatonin levels, plasma TSH levels, plasma glucose levels, and body temperature. Blood sampling is performed at 20-min intervals to clearly delineate the waveshapes of the hormonal patterns. Readings of body temperature and wrist activity are obtained at 10-min intervals using newly available light-weight ambulatory monitoring devices and measures of sleepiness and cognitive performance will be obtained at hourly intervals on the day of blood sampling.

The objectives of these studies are to 1. delineate the phase-shifting effects of bright light immediately following entrainment in humans; 2. determine whether physical activity is a zeitgeber for the human circadian system; 3. provide the basic information necessary to design schedules of bright light exposure and exercise which may facilitate adaptation to abrupt changes of environmental time (e.g. "jet lag") or correct conditions of abnormal circadian timing ( e.g. as associated with sleep disorders, depression or aging); and 4. validate procedures to test phase-shifting effects of behavioral or pharmacological agents in conditions which do not require extended periods of temporal isolation in specially designed units.

## STATUS OF RESEARCH EFFORT

### 1. Pilot studies and adjustments to protocol

In our original proposal, we had planned to purchase ambulatory blood pressure monitors and use the 24-hour variations of heart rate and blood pressure as additional markers of circadian rhythmicity. However, between the time of the preparation of the proposal and the beginning of the grant period, a series of contradicting reports regarding the mechanisms controlling 24-hour changes in heart rate and blood pressure were published. Some of these reports emphasized the circadian nature of the variations, while others proposed that they are primarily controlled by posture changes and/or sleep. To resolve the issue before investing in the equipment, we performed a detailed statistical analysis of a large scale collaborative study designed to delineate the relative roles of sleep and circadian rhythmicity in the control of 24-hour variations in heart rate and blood pressure. Thirty-one healthy young men were studied for a 24-hour period in a standardized physical and social environment using an ambulatory blood pressure monitor (Medilog, Oxford Medical Ltd). Sleep was polygraphically monitored. A best-fit curve based on the periodogram method was used to quantify changes in blood pressure and heart rate over the 24-hour cycle. The typical blood pressure and heart rate patterns were bimodal with a morning acrophase (around 10:00), a small

afternoon nadir (around 15:00), an evening acrophase (around 20:00) and a profound nocturnal nadir (around 03:00). The amplitude of the nycthemeral variations was largest for heart rate (averaging 19.9 % of the mean level), intermediate for diastolic blood pressure (averaging 14.1 % of the mean level) and lowest for systolic blood pressure (averaging 10.9% of the mean level). **Figure 1** shows the mean 24-hour profiles, across all 31 subjects, for systolic blood pressure, diastolic blood pressure and heart rate expressed in absolute value (left panels) or relative values (right panels). The black bars represent the average sleep period. Before awakening, a significant increase in blood pressure and heart rate was already present. Recumbency and sleep accounted for 65 to 75% of the nocturnal decline in blood pressure, but these factors explained only 50% of the nocturnal decline in heart rate. Thus, the combined effects of postural changes and the wake-sleep transition are the major factors responsible for the 24-hour rhythm in blood pressure. In contrast, the 24-hour rhythm of heart rate may reflect an endogenous circadian rhythm, amplified by the effect of sleep. However, the amplitude of the circadian modulation of heart rate is less than 10%, and therefore it was estimated that it would not be a marker of circadian rhythmicity accurate enough to estimate circadian phase positions. Thus, ambulatory blood pressure monitoring was not included in our studies.

During the past year, we have performed studies related to specific aim #1 of our proposal, namely the determination of the magnitude and direction of immediate phase-shifts associated with a single exposure to a 3-hour pulse of either bright light or physical activity. We have slightly modified the experimental protocol proposed in our original application in that instead of having each volunteer participating in four studies, i.e. one baseline and three applications of the same stimulus at different circadian times, we submitted each volunteer to three studies, i.e. one baseline, one with light exposure and one with exercise with the same timing of application of the stimulus. The primary reasons for this modification of the original protocol were to decrease the total duration of the experiments to be performed in the same subject (i.e. from 8-9 weeks to 6 weeks) to avoid costly "drop-outs" and to minimize inter-study variations in daylength and other environmental factors which are seasonally dependent. A schematic representation of the protocol is shown in **Figure 2**.

## 2. Progress on experimental work

So far, we have completed a first series of experiments and have studied 8 normal non-obese men, ages 20-30 years. They were all in good physical condition, none was smoking or taking any drug, none had a personal history of psychiatric or endocrine illness, and all had regular life habits. For each subject, the individual VO<sub>2</sub> max for both legs and arm exercises was determined in the University of Chicago Cardiac Physiology Laboratory using the same exerciser (Schwinn Airdyne) as used during the study. Each study included 5 days of outpatient monitoring and 5 days of inpatient monitoring in the University of Chicago Clinical Research Center. Thus, a total of 120 (i.e. 8 x 3 x 5) outpatient study days and 120 inpatient study days were performed. The stimulus (i.e. bright light of 5,000 lux intensity or exercise) was centered on the time of occurrence of the minimum of body temperature observed under baseline conditions (this ranged between 02:15 and 09:00). For exercise, leg and arm exercise were alternated in 5 cycle

of 36 minutes each, alternating cycles with high and low workloads (starting with the high workload), and including each 15 min of arm exercise, 15 min of leg exercise and 6 min of rest.

During the 5 days of ambulatory monitoring of wrist activity, the volunteers complied with a standard schedule of bedtimes and mealtimes designed individually for each subject, so that it did not represent a significant deviation from usual habits. The volunteers were then admitted to the CRC on a Thursday morning and equipped with an ambulatory temperature monitor. Hourly measures of sleepiness on the Stanford Sleepiness Scale and of cognitive performance (letter cancellation test and digit substitution test) were taken every hour. Meal times and bedtimes were as during the outpatient monitoring period. The subjects were exposed to normal indoor light (<500 lux). During sleep, the subjects were in total darkness. After the second night in the CRC (i.e. on Saturday morning), the subject was served a normal breakfast which was the last meal of the study. Indoor light was kept around 150 lux (i.e. "dim"). Measurement of sleepiness, tests of cognitive performance and monitoring of activity and temperature continued as during the habituation period. At 12:00 noon, a glucose infusion at a rate of 5g/kg/24 hours was started and the subjects remained in bed. No food was allowed. Water and diet decaffeinated sodas were available ad lib. At 15:00, a sampling catheter was inserted in the other arm. At 18:00, blood sampling at 20-min intervals was started and continued non-stop for 38-40 hours, i.e. until 07:00-09:00 on Monday morning. The subjects were continuously sleep-deprived until Monday at 02:00 when lights were turned off and recovery sleep allowed. Wakefulness was monitored by an investigator and the nursing staff at all times during the period of sleep deprivation.

### 3. Results on temperature and activity

**Figure 3** shows the mean profiles of body temperature (top) and wrist activity (bottom) obtained during entrainment, constant routine in the absence of stimulus and recovery sleep. The black bars represent the sleep periods. Following the last sleep period prior to the beginning of constant routine conditions, the volunteers were ambulatory until the time of insertion of the catheter for blood sampling at 15:00. This coincided with a decrease in wrist activity which is clearly evident on **Figure 3**. The amplitude of the rhythm of body temperature was decreased by more than half during the constant routine, i.e. in the absence of sleep, than during entrainment with a normal nocturnal sleep period. The minimum of body temperature also occurred earlier in the absence of sleep than during sleep. Under baseline conditions, the minimum of body temperature occurred between 02:15 (sub#8) and 09:00 (sub#1). The timing of stimulus application during the two following experiments was centered on the minimum of body temperature observed under baseline conditions.

**Figure 4** illustrates the temperature recordings obtained in subject #4 under baseline conditions (top), with application of a 3-hour light pulse (middle) and with application of a 3-hour pulse of exercise (bottom). The timing of application of the stimulus is indicated by a stipled area. The dashed line is a best-fit curve calculated by a robust linear regression algorithm. As apparent on the lower panel, exercise was associated with a marked rise in body temperature. As expected, recovery sleep was associated with a drop in body temperature, masking the endogenous circadian phase

following the constant routine day. Thus, measures of shifts have to be based on other markers.

**Figure 5** shows the mean profiles of wrist activity (top) and temperature (bottom) observed in all 8 subjects during baseline conditions, during exposure to bright light and during exposure to exercise. The timing of application of the stimulus is indicated by a stipled area. To standardize the profiles with respect to inter-individual differences in endogenous circadian phase (as estimated as the minimum of body temperature under baseline conditions), all data are referenced to the time of stimulus presentation prior to calculating the mean curve. This representation clearly shows the constancy of activity levels under baseline conditions and during exposure to light, in contrast to exposure to exercise, which is associated with a marked rise in activity. Approximately two hours after the end of the exercise period, the volunteers were allowed to use the bathroom facilities to wash themselves and this transient increase in activity is also clearly apparent. Light exposure was associated with a slight increase in temperature, which however failed to reach statistical significance. This was probably due to heat irradiated by the light bank. Exercise was associated with an average 1 C rise in body temperature, which occurred over less than 40 minutes. Return to pre-stimulus levels occurred within one hour after the end of the exercise period.

These temperature and activity recordings clearly demonstrate that the experimental techniques and protocol were adequately implemented.

#### 4. Results on glucose and hormones.

A total of approximately 2,800 blood samples have been collected (8 subjects x three studies per subject x 38-40 hours of sample collection x three samples per hour). Glucose, C-peptide and cortisol have so far been measured in duplicate on each sample. Assays for melatonin and TSH are currently under progress.

**Figure 6** shows the mean glucose, C-peptide and cortisol profiles during baseline conditions and during exposure to exercise. During baseline conditions, in the absence of sleep and in the presence of constant levels of activity, the glucose and C-peptide profiles present a low amplitude circadian variation with lower levels during the morning (i.e. approximately 4 hours after timing of stimulus presentation), a progressive rise throughout the day and elevated levels during the evening and nighttime. This variation was present even though glucose was infused at a constant rate throughout the study, indicating that circadian timing influences glucose tolerance, independently of changes in activity level. The well-known wide circadian variation of cortisol levels was observed in all volunteers. Glucose, C-peptide and cortisol profiles obtained during light exposure were essentially identical to those seen during baseline conditions. During exercise exposure, a dramatic drop in glucose and C-peptide levels occurred within 30 minutes. Pre-stimulus levels did not resume for several hours. Effects of exercise on cortisol levels were significant in 5 of the 8 subjects but undetectable in the 3 others. As a result, these effects were not significant at the group level.

Because the constant routine condition was maintained for approximately 20 hours following the end of the exposure to the stimulus, it was possible to observe the circadian rise of cortisol on the first day following exposure to the stimulus. However, in some of the subjects, the circadian rise did not occur before 02:00, i.e. the time when recovery

sleep was allowed. Even when the rise started before 02:00, the accuracy of the estimation was diminished by the interruption of the rise caused by the sleep-related transient inhibition of cortisol secretion. From previous experiments and published data from other groups, it was estimated that the duration of the inhibition of cortisol secretion associated with sleep onset is at least one hour. This estimation was used to correct, whenever necessary (i.e. when the rise occurred after the beginning of recovery sleep), the timing of the cortisol rise. Because of these possible effects of recovery sleep on the estimation of the cortisol rise, results regarding phase-shifting effects of light and exercise that are based on these estimations must be considered as preliminary and subject to confirmation derived from the TSH and melatonin profiles. Indeed, the timing of the circadian rise for these hormones is much earlier than that of cortisol (i.e. between 20:00 and 22:00, instead of 02:00 and 04:00 for cortisol) and thus the "contaminating" effects of recovery sleep will be nonexistent.

Table 1 gives under "basal", the estimations of the timing of the cortisol rise at the beginning of the constant routine, before application of the stimulus. The difference between the basal timing and the timing on the next day, at the end of the constant routine or during recovery sleep, are given under "placebo" for the baseline experiment without stimulus presentation, and under "light" and "exercise" for the two other experiments. On average, placebo was associated with an advance shift of 5 min, while light and exercise were both associated with an advance shift of 51 and 56 min, respectively. Thus, these preliminary results suggest that light and exercise administered during the later part of the subjective night phase advance the circadian clock by roughly one hour. **Figure 7** shows, as an example, the cortisol profiles obtained in Subject #2. The arrow indicates the timing of the circadian cortisol rise, which was advanced by 90 min following exposure to light or exercise, as compared to baseline.

## PUBLICATIONS

Van Cauter, E, Turek FW. Strategies for resetting the human circadian clock (editorial). *New Engl. J Med.* 322: 1306-1307, 1990.

Van Cauter E, van Coevorden A, Blackman JD. Modulation of neuroendocrine release by sleep and circadian rhythmicity. In: *Neuroendocrine Regulation of Reproduction*, SSC Yen and W.W. Vale (eds), Serono Symposia USA, Norwell, Ma,pp 113-122, 1990.

Van Cauter E. Diurnal and ultradian rhythms in human endocrine function : a mini-review. *Horm. Res.* 34: 45-53, 1990.

Van Cauter E. Computer-assisted analysis of endocrine rhythms. In: *Computers in Endocrinology*, V. Guardabasso, D. Rodbard and G. Forti (eds), Serono Symposia Publications, Raven Press, New York, 59-70,1990.

Van Cauter E, Linkowski P, Mendlewicz J. Chronobiologie et Psychiatrie: In:

Acquisitions en Psychiatrie Biologique, J. Mendlewicz (ed), Masson (Paris, France), 1991, in press.

Degaute JP, Van de Borne Ph, Linkowski P, Van Cauter E.  
Quantitative analysis of the 24-hour blood pressure and heart rate patterns in young men. Hypertension, 1991, in press.

In preparation:

Van Reeth O, Blackman JD, Van Cauter E  
Phase-shifting effects of light and exercise in normal man.  
to be submitted to Nature, 1991.

Blackman JD, Van Reeth O, Van Cauter E  
Circadian modulation of glucose regulation and body temperature during constant conditions of activity, posture and caloric intake.  
to be submitted to American Journal of Physiology, 1991

Van Reeth O, Oliner CM, Blackman JD, Polonsky KS, Van Cauter E.  
Effects of exercise on glucose regulation during constant glucose infusion.  
to be submitted to American Journal of Physiology, 1991

## PROFESSIONAL PERSONNEL

Van Reeth, Olivier, M.D.  
Research Associate, 100% effort from March 1, 1990 to February 28, 1991.  
will defend his Ph. D. Thesis in May 1991.

Blackman John D, M.D.  
Fellow in Endocrinology, funded by Endocrinology Training Program at the University of Chicago, will graduate from the Fellowship program on June 30, 1991.

## INTERACTIONS

### 1. Communications at international meetings

Van Reeth O, Blackman JD, Roland D, Van Cauter E  
Phase-shifting effects of a single exposure to light or exercise on the human circadian clock.  
Presented at the 11th European Winter Conference on Brain Research, Crans-Montana, Switzerland, March 1991.

### 2. Consultative and advisory functions by the Principal Investigator

March 1990  
Invited seminar at the Institute of Pharmacology, University of Zürich, Switzerland (Prof. A. Borbely).

*April 1990*

Speaker at the Endocrinology Grand Rounds, Brigham and Women's Hospital, Harvard University, Cambridge, Ma (Prof CA Czeisler).

*May 1990*

Invited speaker at the Ares-Serono Symposium on "Computers in Endocrinology", Milan, Italy.

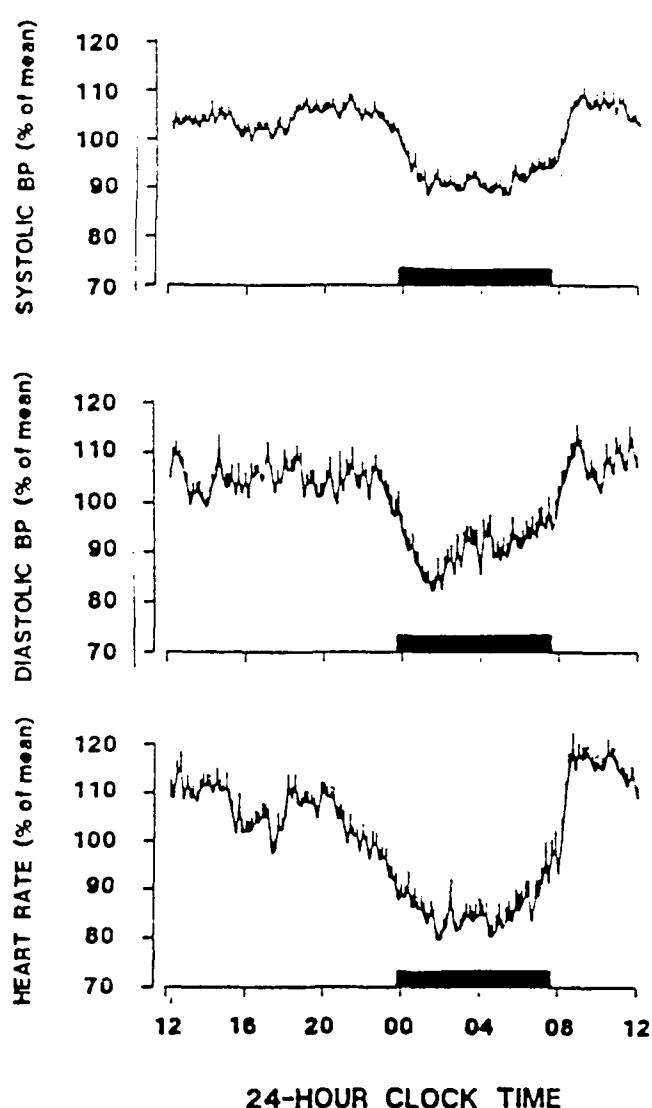
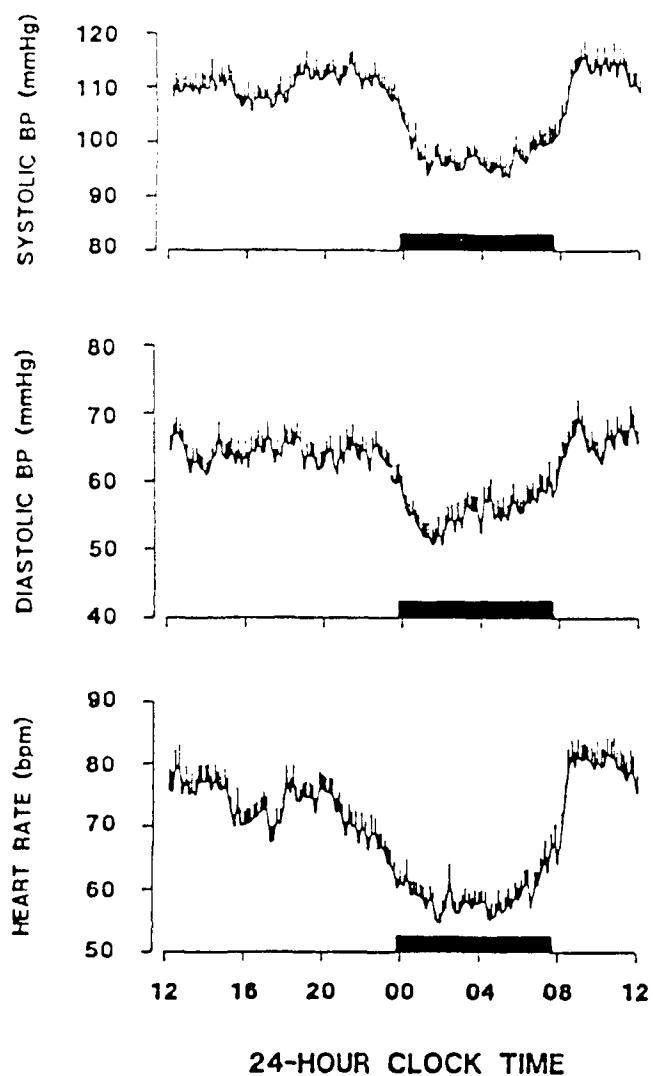
Chairman and organizer of the Symposium on "Interactions between feeding and circadian rhythmicity", Second Meeting of the Society for Research on Biological Rhythms, Amelia Island, Florida.

*February 1991*

Member of the Site Visit reviewing an application for a program project grant on "Sleep, Aging and Circadian Rhythm Disorders" by Dr. C.A. Czeisler, Ph.D. M.D., Harvard Medical School, February 19-20, 1991. Granting Agency: National Institute on Aging, National Institutes of Health.

Invited speaker at the Psychology Colloquium on "Circadian Control of Human Endocrine Profiles" organized on February 13, 1991, at the University of Toronto, Canada.

FIGURE 1



## FIGURE 2

## Protocol For Determination Of Acute Phase - Shifting Effects

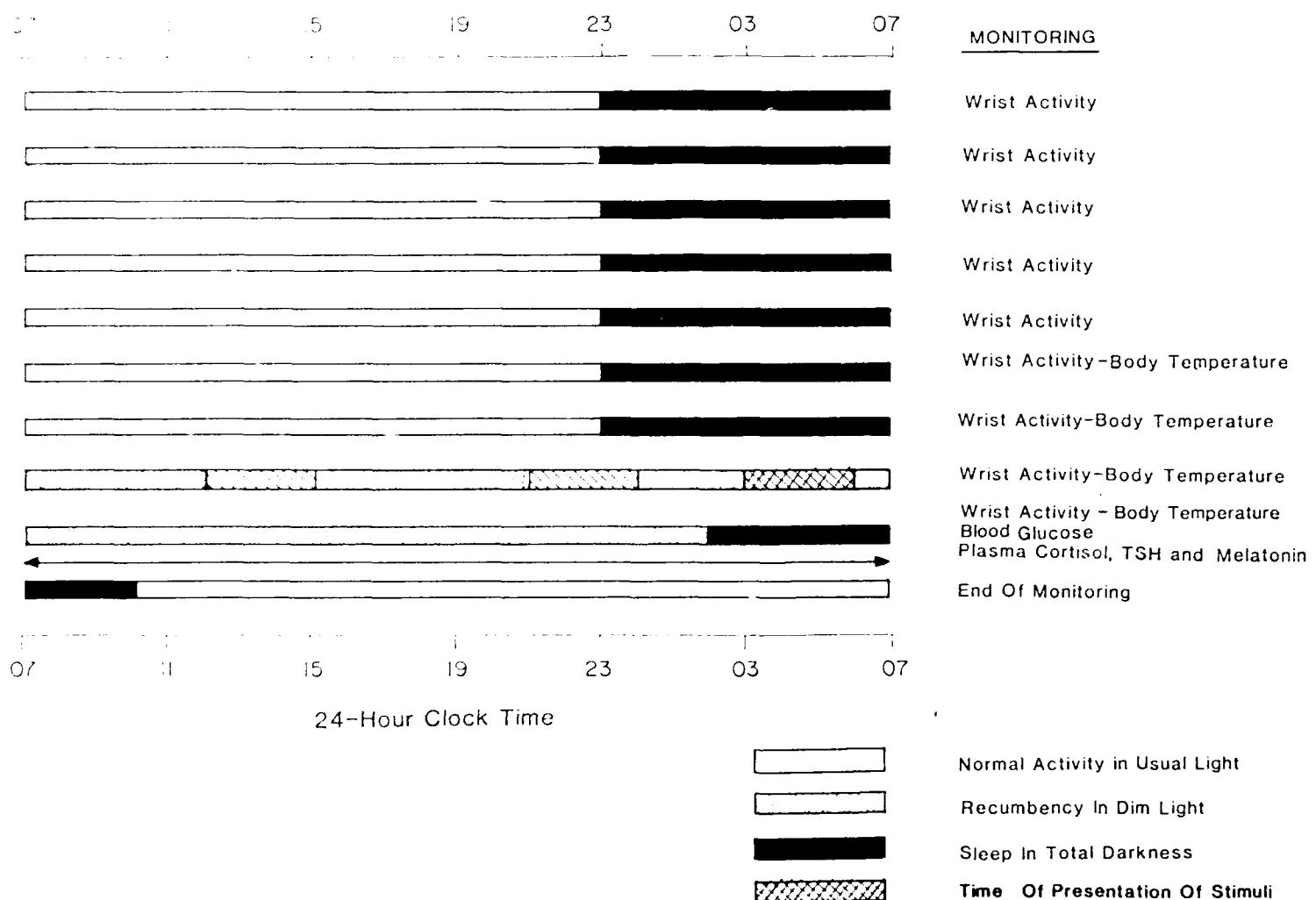
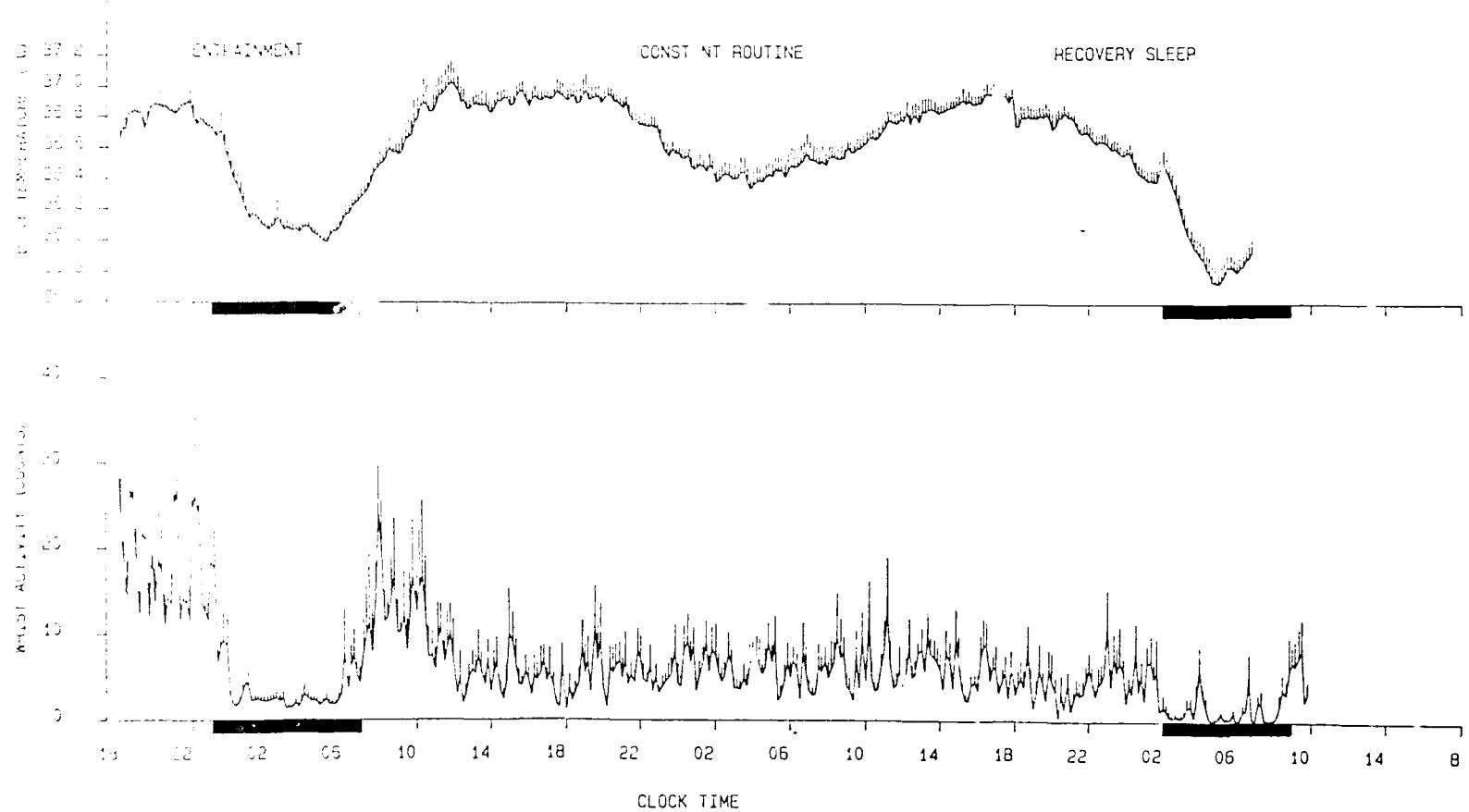


FIGURE 3



**FIGURE 4**

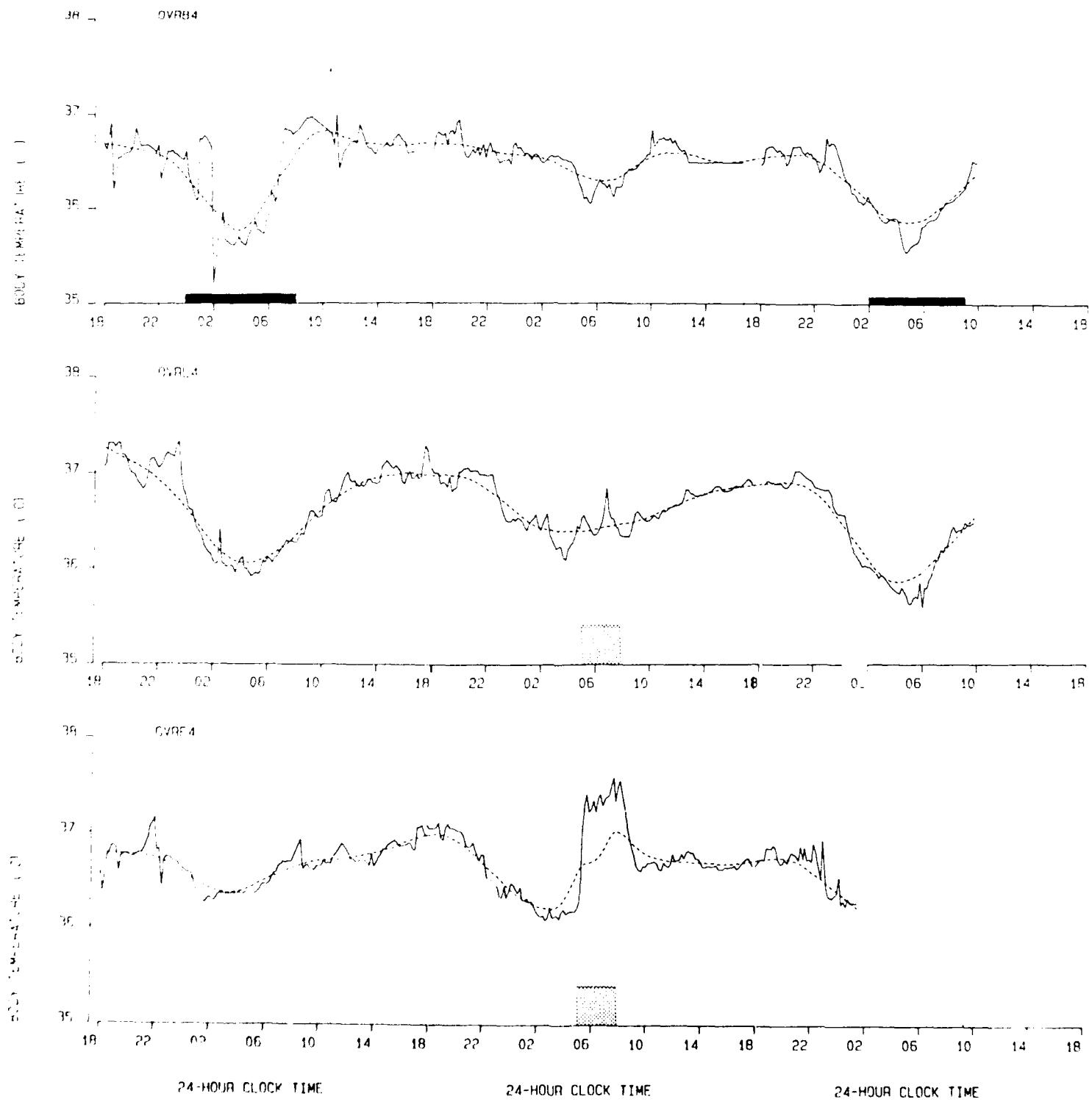


FIGURE 5

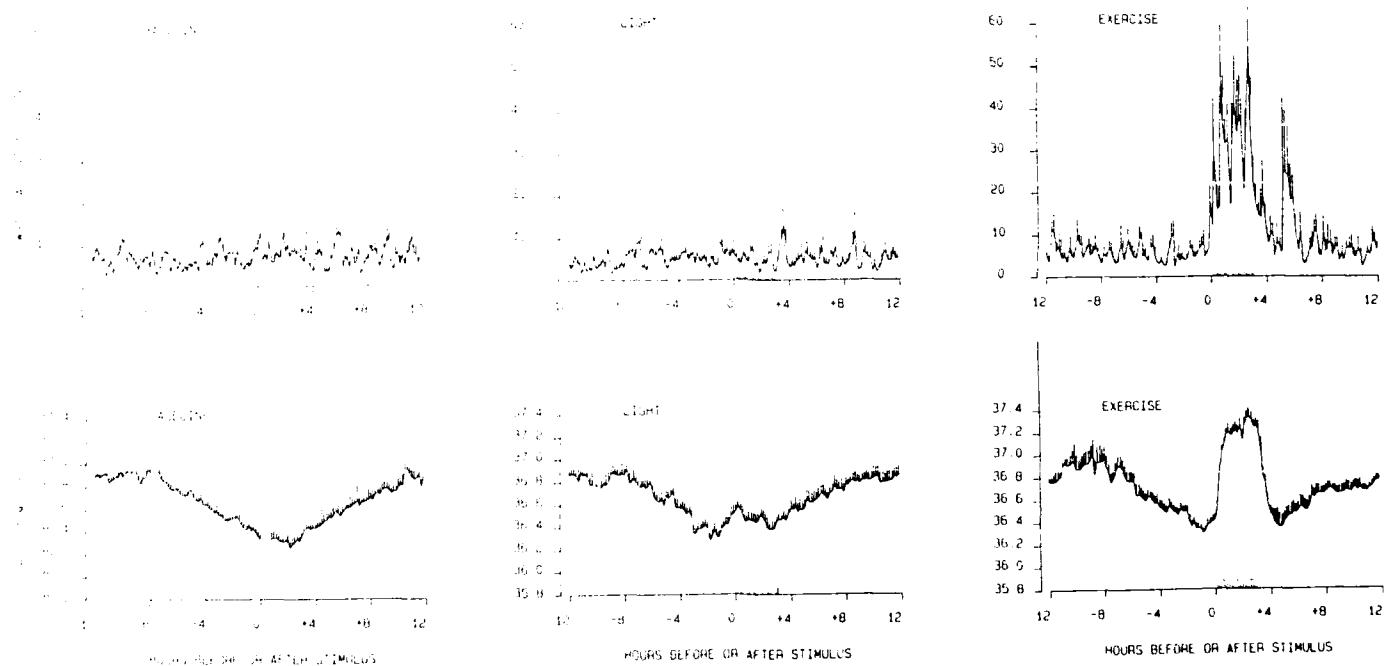
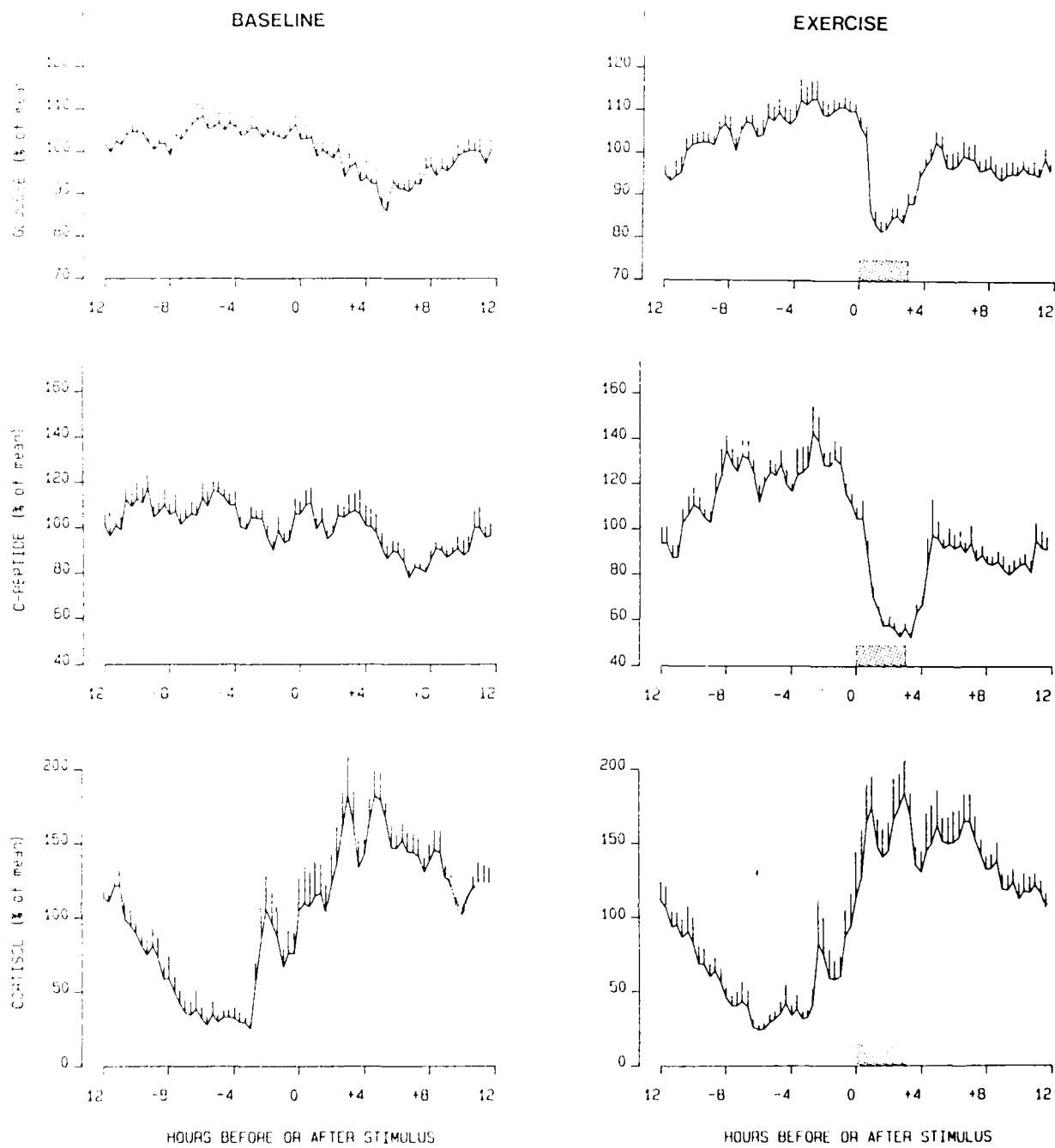
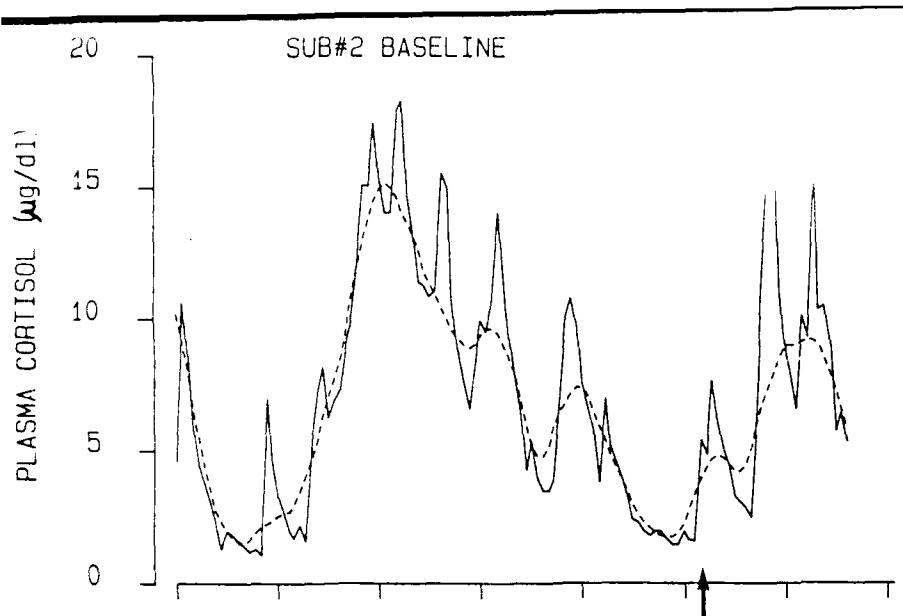


FIGURE 6





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FIGURE 7

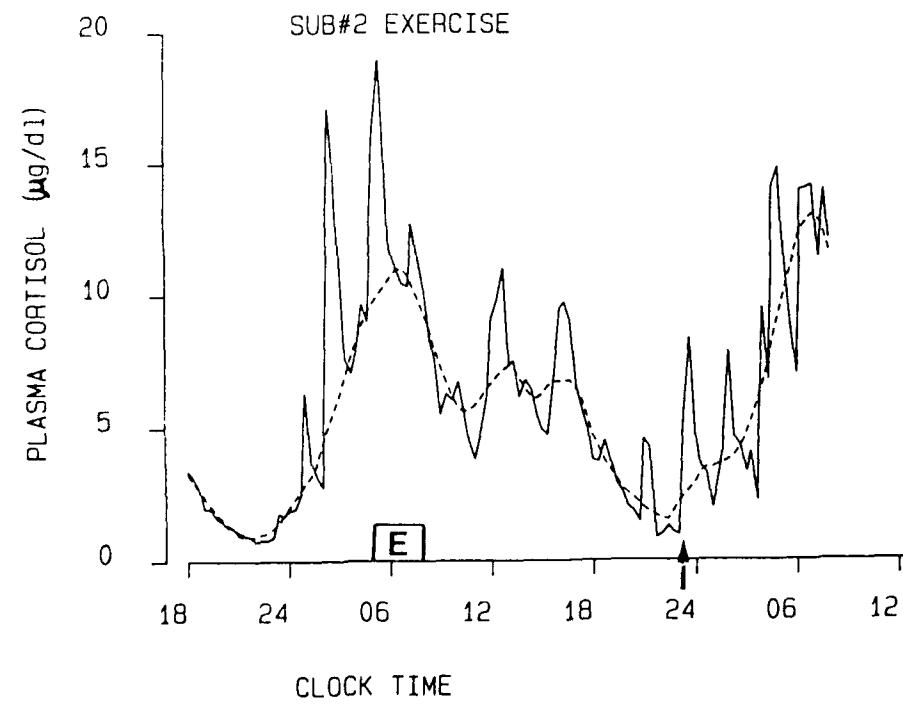
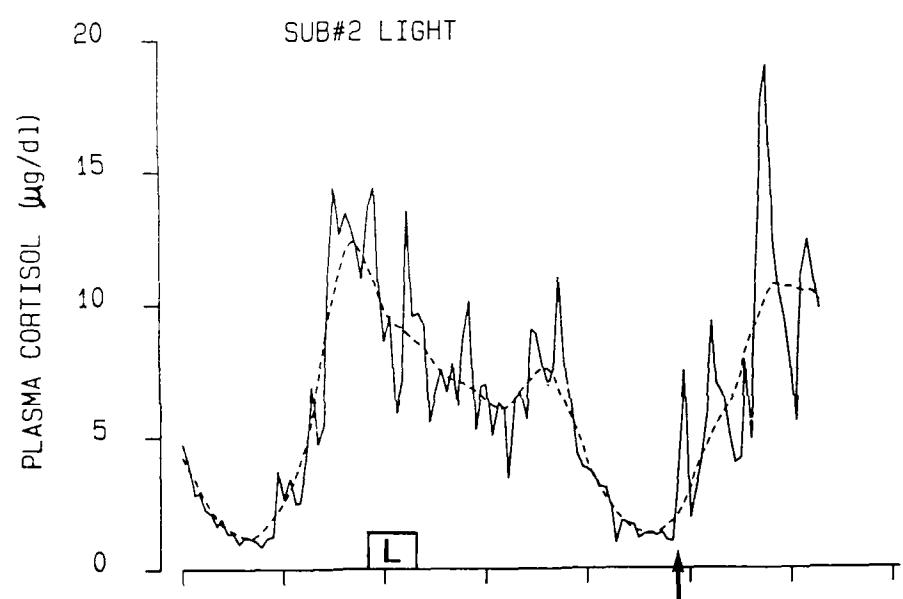


TABLE 1

Phase shifts in cortisol rise compared  
to mean basal values

Subject Nr	Basal (hr)	Placebo (min)	Light (min)	Exercise (min)
1	03:53	113	93	53
2	01:27	27	127	127
3	01:27	47	-13	47
4	00:00	-200	40	120
6	00:07	-73	107	-113
7	02:20	80	80	180
8	01:50	N.A.	10	70
9	02:00	40	-40	-40
Mean ( $\pm$ s.e.m.)	01:38 $\pm$ 24	5 $\pm$ 40	51 $\pm$ 21	56 $\pm$ 33